

DATA EVALUATION RECORD

1. **Chemical:** PP 321/Karate/Lambda - Cyhalothrin
2. **Test Material:** PP321: α -cyano-3-phenoxybenzyl-3-(Z-2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate; a 1:1 mixture of the (1R, 3R, α S) and the (1S, 3S, α R) - esters.-Radio-chemical purity=96.6 and 98.4% MAY 4 12 1501
3. **Study /Action Type:** Daphnia magna life cycle.
4. **Study Identification:** PP321: Daphnia magna. Life cycle study using a flow through system. Submitted by: ICI Americas, Inc. Performed by: ICI Agro Chemicals, Jealotts Hill Research Station, Bracknell, Berkshire, UK.

5. **Reviewed By:**

Candy Brassard
Biologist
Ecological Effects Branch

Signature: Candy BrassardDate: 6/19/906. **Approved By:**

Ann Stavola
Acting Head Section III
Ecological Effects Branch

Signature: Ann StavolaDate: 6/20/90

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7. **Conclusions:** Based on the data submitted, EEB is unable to ascertain if this study is scientifically sound. There are significant discrepancies that need to be addressed. The study authors should refer to section 14 .A for a detailed list of the discrepancies. The primary concerns include: the study authors were unclear as to whether or not the residues reported in the solvent control and the control were technical material or background radiation. In addition, the measured concentrations within the treatment levels varied so much that the guidelines in the 1988 ASTM-1193-87 are not met. The study authors should also be more explicit as to the study method used for delivering the test material and the dilution water within the test vessels.

8. **Recommendations:** The study authors should address the concerns identified in this review.

9. **Background:** This study was submitted to support section 3 registration for cotton.

10. **Discussion of Individual Tests or Studies:**

11. **Materials and Methods:**

A. **Test Organisms:**

The Daphnia magna used were less than 24 hours old at the start of the test. They were taken from cultures maintained at Jealott's Hill in reconstituted water (See Section 2.3), on a diet of yeast and Chlorella vulgaris at approximately 20°C on a 16 hour day. The Daphnia used to produce the young for the test had been cultured separately for over 3 weeks.

B. **Test System:**

Test vessels were 400 ml glass beakers with a capacity of 200 ml to overflow. Hard reconstituted water was pumped to each test chamber at a 1 litre/day using peristaltic pumps. The test chemical was dosed in triethylene glycol, at less than 100 µl/day into dilution water flow using Harvard Apparatus Limited, Model 2265 multiple infusion pumps, fitted with 2 ml glass syringes. The solvent control was dosed with triethylene glycol (same level as treated vessels) and the untreated control with reconstituted water only. Test chambers were maintained at 20±2°C with 1200 lux lighting at water surface for 16 hours per day. Daphnia were fed 0.25 ml Chlorella vulgaris suspension and 0.25 ml active dried yeast suspension two times a day.

C. Study Design:

At study initiation, seven replicates had one single instar Daphnia (A-G) and the three remaining test vessels had five Daphnia each (H-J). Test vessels were arranged in randomized block design.

Every Monday, Wednesday and Friday all chambers were observed for mortality. In chambers A-G, the number of offspring were observed. (The offspring were removed, counted and discarded). If young had been produced in H through J, the young were discarded without counting.

On day 21 the length of surviving adult females were measured using a calibrated graticule lens in a stereo microscope.

Water Quality Characteristics - DO and PH were measured on 0, 7, 14 and 21 days. Temperature was monitored continuously. Water hardness, alkalinity and specific conductivity were observed in 1 control and in 1 highest treatment level on days 5, 12, and 19.

Residue Analysis - excerpted from Submission

The total ^{14}C -PP321 equivalents were determined 3 times a week at each concentration by extraction into hexane. A 50ml water sample was removed by pipette from below the surface of one test chamber at each concentration and extracted with 2 x 5ml hexane. The hexane was taken off, bulked and analyzed by liquid scintillation counting (LSC, see Section 2.6.2.). Once a week, samples were taken from three replicate test chambers at the highest, middle and lowest concentrations.

Characterization of Radioactivity

On study days 0, 7, 14 and 21 samples were taken from representative test concentrations for characterization of the radioactivity. A sample of 1.02 litres was made up from approximately 100ml sampled from each replicate at the test concentration to be analyzed. 2 x 10ml was sampled for LSC (see Section 2.6.2). On study days 0 and 7 the remaining 1 litre sample was extracted with 100ml dichloromethane, acidified with concentrated HCl to approximately pH1 and extracted with a further 100ml followed by 50ml dichloromethane.

The extracts were combined and dried with anhydrous sodium sulphate before taking to dryness by rotary evaporation and then taking up in a small, known volume (100-1000 μ l) for LSC to determine the recovery

over the whole process. An aliquot of the extract was analyzed by TLC (see Section 2.6.2). On study days 14 and 21 the 1 litre water sample was extracted with 2 x 100ml hexane. The extracts were combined and dried with anhydrous sodium sulphate before rotary evaporation and then taking up in a small volume (100 or 200 μ l) of hexane with 2 x 5 μ l taken for LSC. As for the dichloromethane extracts, aliquot were analyzed by TLC and HPLC.

Analytical Methods

Liquid Scintillation Counting (LSC)

Liquid scintillation counting was used to determine the ¹⁴C content of organic and aqueous samples. All samples were mixed with 10ml Optiphase Safe (LKB, Croydon, Surrey, England). Scintillation counting was carried out using an LKB Rackbeta 1219 scintillation counter. The printed results show absolute activity calculated using a quench correcting curve. The suitability of the quench curves for sample types was checked using samples fortified with a known amount of radioactivity. Each group of samples was preceded by control of blank samples and background radioactivity was automatically deducted. All samples were counted for 10 minutes each.

Thin Layer Chromatography

Thin layer chromatography (TLC) was used to analyze the radiochemical purity of the starting ¹⁴C-material using Camlab SIL ^{G25/UV} 254 precoated TLC plates eluted with two solvent systems:-

System 1 - Hexane:diethyl ether 10:1

System 2 - Cyclohexane (saturated with formic acid):diethyl ether 3:2

The concentrated extracts of water (see Section 2.5.2.2) were analyzed using System 2 only. The extracts were chromatographed with an admixed reference marker of unlabelled PP321 (Reference 321/01/04). The marker was visualized on the developed chromatograms by viewing under UV light at 254 nm and the radioactive areas on TLC plates were quantified using a Raytest Rita-68000 Radio TLC Analyzer.

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) was used to analyze the isomeric composition of the starting

¹⁴C-material and the water extracts using the following test system and conditions.

Column : Hichrom Spherisorb S5W length 25cm,
i.d.4.9mm
Pump : Spectra-Physics SP8700 Solvent
Delivery System linked to an SP8750
Organizer.
Mobile Phase: Hexane: Diethyl ether:
Tetrahydrofuran 98:1.0:0.2.
Flow Rate : 2.0ml min⁻¹
Injector : Rheodyne 7125
Detector : Pye-Unicam LC871
Wavelength : 230nm

Samples were fortified with PP564 (Reference 564/01/05) to enable UV detection of the enantiomer pairs. Aliquot of the concentrated water extracts (see Section 2.5.2.2) were then taken to dryness and taken up in 110μl hexane, 2 x 5μl was sampled for LSC and the remaining 100μl analyzed by HPLC. The isomer ratios of the samples were determined by collecting fractions of the eluate corresponding with the resolved PP564 peaks on the HPLC chromatogram, and measuring the radioactivity associated with each fraction by LSC.

D. **Doses:** The following range of nominal concentrations were used: 40, 16, 6.4, 2.56 and 1.024 ng/L, plus a solvent control and a control.

E. **Statistics** - excerpted from submission:

"Survival data from chambers H-J were analyzed by Probit Analysis to obtain estimates of the LC₅₀ using Statistical Analysis Systems (SAS) Version 5.16. Growth and reproduction data from chambers A-G were analyzed by analysis of variance, comparing the treated groups with both the control and the solvent control. The number of young per female reproductive day was calculated by dividing the total young produced by the number of "female reproductive days" for each test chamber. "Female reproductive days" for each test chamber was the number of days survived by the female from day 8, the day reproduction was observed to have started. The maximum number of female reproductive days was therefore 13. Females which died during the study were assumed to have died on assessment days for the purpose of calculating female reproductive days."

12. Reported Results:

The DO levels exceeded 8.3 mg/l, (89% saturation) and the pH was 8.1 to 8.2, water hardness was 165-175 mg/l, alkalinity was 115-125 mg/l and specific conductivity was 555 to 590 μ SCM/l. The water temperature ranged from 19.5 to 23°C. Refer to Table 3 for concentration of PP321 in test vessels. The means measured concentrations for the five PP321 treatments were 19.1, 9.37, 3.50, 1.98 and 0.83 pptr. The flow rate was intended to be 100 ul TEG/day; but the flow rate was observed to be less, therefore measured concentrations were used for results and discussions.

Biological Results: excerpted from submission.

The data from chambers H-J gave LC_{50} values at days 7, 14 and 21 of 8.3, 6.9 and 3.6 ng l^{-1} , respectively (see Table 5). The survival data from chambers A-G is in agreement with these LC_{50} values, with 3 out of 7 Daphnia surviving at day 21 (see Table 11). Analysis of the results from the chambers A-G showed that the total young per chamber and young per female per reproductive day were significantly lower ($P = 0.05$) at mean measured concentrations of 3.50ng l^{-1} and above (See Table 6). The only significant difference ($P = 0.05$) between the lengths of the surviving Daphnia was between the 0.83 ng l^{-1} concentration and the control, however as the 0.83 ng l^{-1} concentration was not significantly different from the solvent control and higher concentrations were not significantly different from the control it was not considered to be a treatment related effect. The no observed effect level (NOEL) was therefore 1.98 ng l^{-1} , based on mean measured concentrations which compares with a NOEL of 2.5 ng l^{-1} , based on nominal concentrations from the previous PP321 D. magna life-cycle study (Reference 3).

The statistical analyses are shown in Tables 5 and 6 and the full results are given in Appendices IV - VI, Tables 9-17.

14. Reviewers Discussion and Evaluation:

A. Test Procedures:

The following discrepancies are noted:

- The study authors were unclear as to the design of the flow through system. Specifically, the design of the delivery of the dilution water to each test vessel/within each treatment level should be described.
- The study authors should have measured residues in all

the test chambers, not just one chamber within each treatment level. Were the samples used for analysis taken from the same vessel each week? The study authors should report in more detail.

- The study authors did not indicate if the test organisms were randomly assigned to the individual test vessels.
- Test chambers should have been conditioned to the chemical so that it would not absorb to test vessels so readily.
- The range of the concentrations with the treatment levels exceeds the criteria recommended in ASTM 1988 - E - 1193-87 Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. These guidelines recommend "in each treatment, the highest measured concentrations obtained during the test in fresh test solutions divided by the lowest must be less than two. The data indicate the individual treatment levels varied by as much as 2.7 to 3.6 times. See below:

Nominal Concentration	Range of Measured Concentration Throughout Study			Range From Low to High
16	4.52	-	14.7	3.25
6.4	1.91	-	5.18	2.7
2.56	1.20	-	3.27	2.72
1.024	0.34	-	1.25	3.6

- EEB evaluated the time weighted averages of the measured concentrations and the results as follows:

Nominal Concentration	ICI's Reported Measured	EPA's Calculated
40	19.1	*
16	9.37	10.08
6.4	3.50	3.54
2.56	1.98	2.10
1.024	0.83	0.84
Solvent Control	0.14	0.128
Control	0.28	0.28

* Could not complete since residue analysis was not completed with the treatment group through Day 21.

- The study authors should indicate why there was such a decrease in the concentrations among all the treatment levels on day 12 of the study. This supports concern for excessive variability.
 - The study authors must indicate if the positive level reported for the control and the solvent control (see Table 3) was parent compound or if it was background radiation. The DPM's should be reported in order for EPA to determine if the numbers reported were significantly different from background and if so, determine if it is test material or not.
 - The recorded daily temperature should be reported. Just the summary of range of temperatures was reported from 19.5 to 23°C. It appears the temperature was higher than recommended, which was perhaps due to the high light intensity which was 1200 lux - where the SEP (EPA-540/9-86-141) recommends 400-800 LUX.
- B. **Statistical Results:** Analysis of variance was conducted in daphnia length, and reproduction (female reproductive days and number offspring per female). Analysis of variance (arc sine transformation) was conducted on survival of the Daphnia in individual test vessels as well as survival in the 3 test vessels with 5 daphnia each per treatment level. The results are as follows:

<u>PARAMETER</u>	<u>RESULTS NG/1 (PPTR)</u>	
Daphnia length	NOEL = 3.5	LOEL = 9.37
No. of young per female	NOEL = 1.98	LOEL = 3.50
No. female reproductive days	NOEL = 3.5	LOEL = 9.37
Adult Survival in individual chambers	NOEL = 1.98	LOEL = 3.5
Adult survival (5 per chamber)	NOEL = 1.98	LOEL = 3.5

C. **Discussion of Results:**

The study authors should respond to the discrepancies noted in Section 14A.

D. Adequacy of Study

- 1) Classification - Not determined at this time.
- 2) Rationale - There are discrepancies, as noted in section 14A that need to be addressed.
- 3) Repairability - Classification is dependent on the adequacy of the data that needs to be submitted.

*entered as supplemental
in database Bpm*

$$TRT=A$$

N Obs	N	Minimum	Maximum	Mean	Std Dev
20	7	13.0000000	13.0000000	13.0000000	0

$$TRT=B$$

N Obs	N	Minimum	Maximum	Mean	Std Dev
20	7	4.0000000	13.0000000	11.7142857	3.4016803

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OUTPUT:

Command ==>

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[illegible]

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[illegible]

OUTPUT
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NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 36 MSE= 15.01587

Number of Means	2	3	4	5	6
Critical Range	4.200	4.415	4.561	4.656	4.735

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	13.000	7	A
A			
A	12.714	7	C

ZOOM

OUTPUT
Command ===>

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General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
A			
A	11.714	7	B
A			
A	11.143	7	D
A			
A	9.286	7	E NOEL
B	4.286	7	F LOEL

NO. OF Female
Repro DAYS
NOMINAL CONC.
NOEL= 6.4 ng/l
LOEL= 16.0 ng/l

No Female
Repro. Days

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Analysis Variable : RESP

ZOOM R

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		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
O	T	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
B	T	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
S	T	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
1	A	83	75	92	75	75	75	77
2	B	81	80	70	72	11	68	92
3	C	91	105	88	65	86	81	76
4	D	79	77	74	59	65	76
5	E	14	48	29	40	33	26
6	F	2	12	0	8	0	17	0

ZOOM

OUTPUT
Command ==>

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General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	6	A B C D E F

Number of observations in data set = 120

NOTE: Due to missing values, only 40 observations can be used in this analysis.

ZOOM

OUTPUT

Command ===>

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General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	33124.39405	6624.87881	33.94	0.0001
Error	34	6636.38095	195.18768		
Corrected Total	39	39760.77500			
	R-Square	C.V.	Root MSE	RESP Mean	
	0.833092	24.54275	13.97096	56.9250000	

ZOOM

OUTPUT

Command ===>

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General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	33124.39405	6624.87881	33.94	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	33124.39405	6624.87881	33.94	0.0001

ZOOM

OUTPUT

Command ===>

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General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 34 MSE= 195.1877
 WARNING: Cell sizes are not equal.
 Harmonic Mean of cell sizes= 6.631579

Number of Means 2 3 4 5 6
 Critical Range 15.59 16.38 16.93 17.28 17.57

Means with the same letter are not significantly different.

ZOOM

OUTPUT

Command ==>

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General Linear Models Procedure

Duncan Grouping		Mean	N	TRT
NO. OF YOUNG PER FEMALE	A	84.571	7	C
	A			
	A	78.857	7	A Control
	A	71.667	6	D) NOEL 2.56 ng/l
	A			
NOMINAL CONC.	A	67.714	7	BSolvent Control
	B	31.667	6	E) LOEL 6.4 ng/l
	C	5.571	7	F) NOMINAL CONC.

ZOOM

OUTPUT

Command ==>

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Analysis Variable : RESP

TRT=A

N Obs	N	Minimum	Maximum	Mean	Std Dev
20	7	75.0000000	92.0000000	78.8571429	6.4917530

TRT=B

N Obs	N	Minimum	Maximum	Mean	Std Dev
20	7	11.0000000	92.0000000	67.7142857	26.3230842

ZOOM

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Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	54176.78713	9029.46452	9.11	0.0001
Error	42	41623.62914	991.03879		
Corrected Total	48	95800.41627			
	R-Square	C.V.	Root MSE		EFFECT Mean
	0.565517	59.12561	31.48077		53.2438755

OUTPUT
Command ===>

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General Linear Models Procedure

Dependent Variable: EFFECT

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	6	54176.78713	9029.46452	9.11	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	54176.78713	9029.46452	9.11	0.0001

OUTPUT
Command ===>

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General Linear Models Procedure

Duncan's Multiple Range Test for variable: EFFECT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 42 MSE= 991.0388

Number of Means	2	3	4	5	6	7
Critical Range	33.96	35.70	36.87	37.67	38.32	38.85

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	89.96	7	A
A	77.11	7	B

OUTPUT
Command ===>

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General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
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A

A

77.11

7

C

A

A

77.11

7

D

NOEL

B

38.56

7

E

LOEL

B

B

12.85

7

F

0.00

7

G

Nominal
 NOEL = 2.56 mg/l
 LOEL = 6.4 mg/l

C
C
C

ADULT SURVIVAL
 IN
 TEST VESSELS
 A-G (7 test vessels)

ZOOM R

OUTPUT

Command ==>

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General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
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A

A

77.11

7

C

A

A

77.11

7

D

B

38.56

7

E

B

B

12.85

7

F

C
C
C

0.00

7

G

ZOOM R

OUTPUT

Command ==>

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General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
-----------------	------	---	-----

A

A

77.11

7

C

A

A

77.11

7

D

ZOOM

OUTPUT

Command ==>

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General Linear Models Procedure

Duncan Grouping		Mean	N	TRT
	A	3.5100	6	D
	A			
	A	3.5086	7	A
	A			
	A	3.5000	3	E
	A			
	A	3.4767	6	B
	A			
B	A	3.3450	6	C
B				
B				
B				
B		3.2800	1	F

ZOOM

OUTPUT

Command ==>

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		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
O	T	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
B	R	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
S	T	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
1	A	100	60	80
2	B	100	80	100
3	C	80	100	100
4	D	100	80	100
5	E	60	40	40
6	F	0	0	0
7	G	0	0	0

ZOOM R

OUTPUT

Command ==>

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OBS	TRT	RESP	ARS	EFFECT
1	A	100	1.57080	89.9638
2	A	60	0.88608	50.7481
3	A	80	1.10715	63.4094
4	A	.	.	.
5	A	.	.	.
6	A	.	.	.
7	A	.	.	.
8	A	.	.	.
9	A	.	.	.
10	A	.	.	.
11	A	.	.	.
12	A	.	.	.
13	A	.	.	.
14	A	.	.	.
15	A	.	.	.
16	A	.	.	.
17	A	.	.	.

ZOOM R

OUTPUT
Command ==>

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OBS	TRT	RESP	ARS	EFFECT
18	A	.	.	.
19	A	.	.	.
20	A	.	.	.
21	B	100	1.57080	89.9638
22	B	80	1.10715	63.4094
23	B	100	1.57080	89.9638
24	B	.	.	.
25	B	.	.	.
26	B	.	.	.
27	B	.	.	.
28	B	.	.	.
29	B	.	.	.
30	B	.	.	.
31	B	.	.	.
32	B	.	.	.
33	B	.	.	.
34	B	.	.	.

ZOOM R

OUTPUT
Command ==>

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OBS	TRT	RESP	ARS	EFFECT
35	B	.	.	.
36	B	.	.	.
37	B	.	.	.
38	B	.	.	.
39	B	.	.	.
40	B	.	.	.

41	C	80	1.10715	63.4094
42	C	100	1.57080	89.9638
43	C	100	1.57080	89.9638
44	C	.	.	.
45	C	.	.	.
46	C	.	.	.
47	C	.	.	.
48	C	.	.	.
49	C	.	.	.
50	C	.	.	.
51	C	.	.	.

ZOOM R

OUTPUT

Command ==>

SAS 13:06 Wednesday, June 6, 1990 14				
OBS	TRT	RESP	ARS	EFFECT
52	C	.	.	.
53	C	.	.	.
54	C	.	.	.
55	C	.	.	.
56	C	.	.	.
57	C	.	.	.
58	C	.	.	.
59	C	.	.	.
60	C	.	.	.
61	D	100	1.57080	89.9638
62	D	80	1.10715	63.4094
63	D	100	1.57080	89.9638
64	D	.	.	.
65	D	.	.	.
66	D	.	.	.
67	D	.	.	.
68	D	.	.	.

ZOOM R

OUTPUT

Command ==>

SAS 13:06 Wednesday, June 6, 1990 15				
OBS	TRT	RESP	ARS	EFFECT
69	D	.	.	.
70	D	.	.	.
71	D	.	.	.
72	D	.	.	.
73	D	.	.	.
74	D	.	.	.
75	D	.	.	.
76	D	.	.	.
77	D	.	.	.
78	D	.	.	.
79	D	.	.	.
80	D	.	.	.
81	E	60	0.88608	50.7481
82	E	40	0.68472	39.2157

83	E	40	0.68472	39.2157
84	E	.	.	.
85	E	.	.	.

ZOOM R

OUTPUT
Command ==>

		SAS	13:06 Wednesday, June 6, 1990 16		
OBS	TRT	RESP	ARS	EFFECT	
86	E	.	.	.	
87	E	.	.	.	
88	E	.	.	.	
89	E	.	.	.	
90	E	.	.	.	
91	E	.	.	.	
92	E	.	.	.	
93	E	.	.	.	
94	E	.	.	.	
95	E	.	.	.	
96	E	.	.	.	
97	E	.	.	.	
98	E	.	.	.	
99	E	.	.	.	
100	E	.	.	.	
101	F	0	0	0	
102	F	0	0	0	

ZOOM R

OUTPUT
Command ==>

		SAS	13:06 Wednesday, June 6, 1990 17		
OBS	TRT	RESP	ARS	EFFECT	
103	F	0	0	0	
104	F	.	.	.	
105	F	.	.	.	
106	F	.	.	.	
107	F	.	.	.	
108	F	.	.	.	
109	F	.	.	.	
110	F	.	.	.	
111	F	.	.	.	
112	F	.	.	.	
113	F	.	.	.	
114	F	.	.	.	
115	F	.	.	.	
116	F	.	.	.	
117	F	.	.	.	
118	F	.	.	.	
119	F	.	.	.	

ZOOM R

OUTPUT
Command ==>

OBS	TRT	RESP	ARS	EFFECT
120	F	.	.	.
121	G	0	0	0
122	G	0	0	0
123	G	0	0	0
124	G	.	.	.
125	G	.	.	.
126	G	.	.	.
127	G	.	.	.
128	G	.	.	.
129	G	.	.	.
130	G	.	.	.
131	G	.	.	.
132	G	.	.	.
133	G	.	.	.
134	G	.	.	.
135	G	.	.	.
136	G	.	.	.

ZOOM R

OUTPUT

Command ==>

General Linear Models Procedure

Duncan's Multiple Range Test for variable: EFFECT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 14 MSE= 164.2884

Number of Means	2	3	4	5	6	7
Critical Range	22.40	23.49	24.24	24.64	24.94	25.17

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	81.11	3	C
A	81.11	3	B

ZOOM R

OUTPUT

Command ==>

General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
A	81.11	3	D

Duncan

A			
A	68.04	3	A
B	43.06	3	E
C	0.00	3	F
C			
C	0.00	3	G

ZOOM R

OUTPUT
Command ==>

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General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
A			
A	81.11	3	D
A			
A	68.04	3	A NOEL
B	43.06	3	E LOEL
C	0.00	3	F
C			
C	0.00	3	G

Handwritten notes:
SURVIVAL H-J
NOMINAL CONC.
2.56 ng/l
6.4 ng/l

Handwritten notes on right:
Dose
H-J

ZOOM R

OUTPUT
Command ==>

SAS 13:06 Wednesday, June 6, 1990 24

General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
A			
A	81.11	3	D
A			
A	68.04	3	A
B	43.06	3	E
C	0.00	3	F
C			
C	0.00	3	G

Dependent Variable: RESP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.14972750	0.02994550	2.23	0.0859
Error	23	0.30896905	0.01343344		
Corrected Total	28	0.45869655			
R-Square		C.V.	Root MSE	RESP Mean	
0.326420		3.350123	0.115903	3.45965517	

ZOOM

OUTPUT

Command ==>

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General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	0.14972750	0.02994550	2.23	0.0859
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	0.14972750	0.02994550	2.23	0.0859

ZOOM

OUTPUT

Command ==>

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General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 23 MSE= 0.013433
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 3.036145

Number of Means 2 3 4 5 6
Critical Range 0.194 0.204 0.211 0.215 0.218

Means with the same letter are not significantly different.

ZOOM

OUTPUT
Command ==>

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General Linear Models Procedure

Duncan Grouping		Mean	N	TRT
<i>Daphnia</i> <i>Length</i>	A	3.5100	6	D
	A			
	A	3.5086	7	A-control <i>Daphnia</i> <i>Growth</i> A-G
	A			
	A	3.5000	3	E
	A			
	A	3.4767	6	B-control
	A			
	A	3.3450	6	C
	A			
B				
B				
B				
B				
B				
B		3.2800	1	(F) LOEL?

ZOOM

OUTPUT
Command ==>

SAS 13:06 Wednesday, June 6, 1990 5

General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 23 MSE= 0.013433

WARNING: Cell sizes are not equal.

Harmonic Mean of cell sizes= 3.036145

Number of Means 2 3 4 5 6
Critical Range 0.194 0.204 0.211 0.215 0.218

Means with the same letter are not significantly different.